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TRANSTUBULAR LEAKAGE OF GLOMERULAR FILTRATE IN HUMAN ACUTE RENAL FAILURE AN IN-VIVO STUDY

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TRANSTUBULAR LEAKAGE OF GLOMERULAR FILTRATE IN HUMAN ACUTE RENAL FAILURE. AN IN-VIVO STUDY

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NOTE: The laboratory work of this thesis was carried out at Stanford University School of Medicine, Division of Nephrology, Department of Medicine, during summer 1978 when the author was visiting that institution. The results of the work subsequently was published in the article

Transtubular leakage of glomerular filtrate in human acute renal failure.

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I. Introduction

Over the past several decades, the general topic of acute renal failure has continued to attract interest and stimulate investigation. This is, in part, a reflection of the many clinical entities that can result in an acute renal debacle and an incomplete understanding of their role in the production of the renal lesion. Despite intensive effort of investigation, the pathophysiology of nonspecific* acute renal failure (ARF) is still uncertain. While some investigators emphasize tubular necrosis, others see vasoconstriction as the principal cause of renal failure. Both animal models and studies of patients exist. The animal models have the advantage that extensive data are available from direct technics such as micropuncture. Although evidence from studies of patients is fragmentary and indirect, it is worth attention because of the uncertain relevance of animal models to human acute renal failure, especially the common post-ischemic type.

*Acute renal failure from specific causes, such as glomerulonephritis, vasculitis and interstitial nephritis etc., are not considered in this thesis.

A. Observations in animal models

Acute renal failure (ARF) has been induced in rats and dogs with a number of models: (1) nephrotoxins such as mercury, dichromate and uranyl nitrate; (2) pigments including myoglobinuria from muscle injury with glycerol, hemoglobinuria and methemoglobinuria; (3) ischemia induced by temporal occlusion of the renal artery, and by intrarenal infusion of vasoconstrictors. From these models, several mechanisms have been postulated to account for the oliguria in ARF : (1) passive back diffusion of the glomerular filtrate through a damaged tubular epithelium; (2) obstruction of the tubules by casts or edema; (3) a severe reduction in glomerular filtration rate due to such causes as preglomerular vasoconstriction; a decrease in glomerular plasma flow; or an alteration in the ultrafiltration capillary membranes (K_f). The situation becomes particularly complex in several of those experimental models in which more than one abnormality exist.

1. Leakage of Filtrate across damaged tubular epithelium

According to the "passive back flow" theory of the pathophysiology of ARF(77), glomerular filtrate is formed at a normal or only slightly reduced rate but is quantitatively reabsorbed, quite passively, across injured epithelium which can no longer serve as an effective absorption barrier. This theory has enjoyed wide acceptance in the past and is still frequently cited as a major determinant of renal insufficiency.

Among the earliest proponents of tubular injury as the basis for oliguric renal failure, Richards(77) reported in 1929

that in the anuric stage of cyanide or mercury poisoning in frogs, flow of fluid from the glomerulus into the beginning of the proximal tubule was "normal or even increased." That brief report, however, gave no information as to the dose of mercury used, the means of determining the rate of glomerular filtration (GFR), the number of animals studied, the time after injection that studies were performed or whether histologic alterations had indeed been produced. That study did not receive much attention. In contrast, Oliver's elegant microdissections(69,70) in the 1950's documenting structural evidence of "disruptive" or "tubulorrhextic" damage to nephrons of animals and/or patiente with nephrotoxic and circulating ischemic insults. On the basis of his findings, Oliver suggested that backleak of glomerular filtrate across damaged tubular epithelium accounts for both the oliguria and the renal interstitium edema found in these animals or patients.

That this pathophysiological event occurs in experimental ARF in the rat has subsequently been demonstrated by micropuncture techniques. Some investigators have used such nephrotoxic chemicals as mercuric chloride or uranyl nitrate as the etiologic agent of the renal failure, others have involved the use of heme pigments, and still others have employed prolonged total renal ischemia. Of all these studies published thus far, only a few have incriminated strongly the "passive backflow" theory as the major etiological factor in ARF; these few studies will be discussed below in more details.

Bank, Mutz and Aynedjian(8) performed renal micropuncture in rats 24 hours after they were given subcutaneous injections of low doses of mercuric chloride (3.0-3.5mg/kg),

at a time when almost all animals became anuric. They found that intravenously injected lissamine green, which normally becomes deeper in color as it traverses the proximal tubule (the normal tubule does not reabsorb the dye at all), appeared in some proximal tubules of these rats, but became more pale with the passage of time. The dye failed to appear at all in distal tubule segments visible on the kidney surface. The authors interpreted this finding, quite logically, as significant leakage of the dye across damaged proximal tubule epithelium. In the same study, Bank and co-workers measured the single nephron tubular fluid to plasma inulin $(TF/P)_{IN}$ ratios using micropuncture, and from that, inulin clearance at three sites: early proximal tubule, late proximal tubule, and distal tubule. Inulin clearance was found to be normal at the beginning of the proximal tubule but fell by more than 60% by the late proximal convolutions. It is interesting to note that the upper 2/3 of the proximal tubule despite this fact showed no disruption on light microscopy 24 hours after the low dose of mercury used. Furthermore, the suggested "leakage" of 2/3 of the filtered inulin from this early proximal tubule became more remarkable when only 29% of the inulin remaining was reported to be lost from the distal one-third of the proximal tubule, the only segment of the nephron displaying frank tubular necrosis.

Another micropuncture study supporting the passive backflow theory was published by Eisenbach and Steinhausen(87), who reported that an unspecified proportion of inulin infused into individual proximal tubules of one kidney in mercury poisoned rats appeared in the ureteral urine collected from

both kidneys. This could only occur if the injectate had leaked from the tubule into which it was injected, recirculated and then was filtered.

Increased inulin permeability has also been noted in the uranyl nitrate model in both dog and rat. In an elegant study published by Blantz(11), low dose (15mg/kg) and high dose (25mg/kg) of intravenous uranyl nitrate (UN) were administered to Munich-Wistar rats. Using ^{14}C-inulin and the micropuncture technique, the (1) total kidney GFR; (2) single nephron gfr (sngfr) at the early vs. late proximal tubule were determined in both groups of rats, as well as in a control group. In the same study, micro-injection of ^{3}H-inulin and ^{14}C-mannitol into proximal tubules were performed on a separate control and high dose (25mg/kg) UN group. Recovery of both isotopes were measured from the microinjected as well as the contralateral kidneys. Total kidney GFR was reduced to 47% of control in the low dose group and to 21% in the high dose group. The simultaneous single nephron gfr (sngfr) measured at the early proximal tubule was found to be essentially identical in the control and low-dose groups and reduced only to 63% (statistically significant) of control in the high dose group. This disparity in UN effect upon GFR and sngfr, with a greater reduction in GFR than the corresponding sngfr, was thought to be due to tubular back-diffusion of solute through damaged epithelium distal to the early proximal tubule, as demonstrated by microinjection of inulin and mannitol in the proximal tubule. Recovery of ^{3}H-inulin and ^{14}C-mannitol from the microinjected kidney was nearly complete at 96±1 and 95±2% respectively, in

control rats. However, in the high dose UN treated group, recovery of inulin and mannitol from the microinjected kidney was 55±10 and 43±8%. Also, significant radioactive material was excreted from the contralateral kidney at 7±3 and 12±2%, respectively. Total radioactivity excreted from both kidneys was less than 100% after UN because of the very low GFR bilaterally, which permitted significant quantities of inulin and mannitol, which had leaked from the tubule, to leave the plasma volume. In addition, comparision of sngfr using ^{14}C-inulin, measured in early vs. late proximal tubule revealed no difference after high dose UN, suggesting no significant leak of inulin from the early proximal tubule, and that the decreased sngfr (63% of control) at that site was due to primary reductions in ultrafiltration. A detailed analysis of the separate factors which can affect the sngfr showed that total glomerular permeability (L_pA) was reduced to 53% of control in the low dose group and to 26% of control in the high dose group. The conclusions of this study were twofold; namely UN decreases GFR by two mechanisms: (a) tubular damage leading to back-diffusion of solutes and (b) a primary reduction in sngfr due to reduced glomerular permeability in high dose UN.

Passive backflow has also been suggested in renal ischemia. Arendshorst et al.(2) injected small volumes of lissamine green into the proximal tubule of animals 22-26 hours after an hour of renal artery clamping. Although intratubular pressure never increased more than 2 mm Hg, the dye was seen to exit from the tubule creating a "halo" effect in the interstitium. This phenomenon did not occur in normal tubules. One could possibly still criticize

this study by suggesting that the small increase in intratubular pressure altered permeability in an otherwise friable tubule. However, a recent study by Donohoe et al.(31) has obviated that possible criticism and clearly shown that tubular permeability is increased in the clamp model. Horseradish peroxidase (HRP) (molecular weight 40,000 and Einstein-Stokes radius (ESR)=30Å), a probe molecule that can be cytochemically demonstrated in cells or tissue by light and electron microscopy, was dissolved in normal saline and was microinjected into both control and 60-min. ischemia damaged rat nephrons. This was followed by tissue fixation using glutaraldehyde in sodium cacodylate buffer. Thereafter, cortical slices of excised kidneys were immersed in the same fixative for 3 more hours, washed overnight in cold sodium cacodylate buffer, and reacted for peroxidase with specific reagents. Following incubation, the purified sections were postfixed in osmium tetroxide for 90 minutes, dehydrated, imbedded in Epon, and looked under light and electron microscopy. To circumvent the criticism that microinjection per se, by raising intratubular pressure, may artifically cause transepithelial leakage of tracer molecules, the authors repeated the experiment except for using microinjection of the tracer molecule, they used intravenous injection of HRP into 3 rats 1 to 2 hours after 60 minutes of renal ischemia, and into 2 rats 1 to 2 hours after 25 minutes of ischemia. A control rat was similarly injected. The kidneys were then fixed within 5 minutes of injection by intravenous perfusion of the same fixative, and the same procedures repeated as mentioned above. Results indicated that in the proximal convoluted tubules subjected to 60 minutes of ischemia, <u>both</u> the

microinjected and the i.v. injected HRP penetrated diffusely through the cytoplasm of isolated tubular cells and out into the interstitium and capillaries. In contrast, in control proximal tubule and in convoluted proximal tubule subjected to 25 minutes of ischemia, epithelial cells excluded peroxidase from their cytoplasmic matrix, the internal distribution of the tracer being confined to phagolysosomes. That such a large protein can so readily travel from lumen to interstitium clearly indicates that these cells had lost their selective permeability. In the same study, 35% of ^{14}C-inulin microinjected into ischemic proximal convoluted tubule was recovered in contralateral urine after 60 minutes of ischemia; 11% was recovered after 25 minutes; thus providing further evidence of tubular backleak of filtrate into the interstitium and circulation.

2. Tubular Obstruction

There is no question that tubular obstruction is an important pathophysiologic mechanism in various forms of ARF. This is especially convincing in the renal artery clamping model. Arendshorst and co-workers(2) found a marked increase in intratubular pressure 1-3 hours after release of total renal artery occlusion. Immediately after removal of the occluding clamp, RBF rapidly returned to about 50% of normal and the previously collapsed proximal convolutions became filled with fluid, indicating the resumption of glomerular filtration. However, the proximal intratubular pressure rose from 11 to 31 mm Hg, and the distal intratubular pressure was also significantly elevated. By 22-26 hours after occlusion, intratubular pressures had returned to essentially control level. However, the authors suggested that this may be due to a decreased GFR caused by

increased proglomerular vascular tone, and not necessarily relief of obstruction. Comparable results have been found by Tanner and Sophason(89). Furthermore, these latter investigations performed microdissection studies which consistently demonstrated the presence of casts in the distal tubule of post-clamp but not control animals. Tanner and Steinhausen(90) also examined the pressure-flow characteristics of tubule from normal and post-renal artery clamp animals. In the majority of ARF tubules, microperfusion at a rate of 15 nl/min led to an immediate and marked increase in intratubular pressure to values as high as 150 mm Hg followed by a spontaneous fall. The spontaneous fall was associated with the appearance of the perfusion fluid in the distal tubule. This series of events was never seen in tubules of normal animals. The authors concluded that tubular obstruction was <u>initially</u> present and that the marked increase in intratubular pressure led to venting of the tubule.

The basis of this obstruction has recently been studied by Donohoe and associates(31). Utilizing histological techniques, they found that more than 90% of straight proximal tubules were occluded by swollen blebs of desquamated proximal tubular microvilli.

Tubular obstruction has also been noted in some, but not all, of the other models of ARF under discussion. Burke et al.(15) found a normal to increased intratubular pressure in association with a marked fall in nephron GFR in the initial phase of norepinephrine induced ARF in the dog. Previous studies have suggested that obstruction is not present in the glycerol model in view of the low intratubular pressure(67). However, a recent study by O'Connor et al.(85) have found that intratubular pressure is

uniform and normal from 3 to 18 hours after glycerol despite a marked reduction in GFR which should tend to decrease intratubular pressure. Superficial proximal tubules are not dilated and casts are usually not seen in the surface nephrons. After Ringer loading, however, a modest amount of cellular debris is readily seen traversing the collecting duct system and exiting from the duct of Bellini. It seems possible that this debris may cause partial obstruction to flow in some portion of the distal nephron in circumstances in which urine flow is low. Alternative explanations for this finding are certainly possible.

In rats made oliguric with mercury, dichromate or methemoglobin(8,45,54), micropuncture studies show that many nephrons have elevated intratubular pressure. In ARF due to methemoglobin, the pigment itself is deposited(54). Flamenbaum and associates(37) found significant evidence for tubular obstruction in the mercuric chloride model in the rat. Normal tubular pressure coupled with a markedly reduced nephron GFR was demonstrated. When the tubule was vented, GFR was restored to normal levels. In contrast, no evidence for tubular obstruction has been found in the uranyl nitrate model(11,86).

In addition to the obvious mechanical effects of tubular obstruction, Arendshorst and co-workers(3) have suggested a secondary hemodynamic effect of this alteration. Complete ureteral ligation of 24 hours duration markedly decreased glomerular capillary pressure estimated from the stop-flow pressure method. A similar response also occurred in tubules from normal kidneys in which an oil-block had been placed for 24 hours. They suggested that tubular obstruction eventually led to afferent arteriolar constriction with a consequential fall in intra-

tubular pressure. This phenomenon would seemingly explain the eventual reduction in intratubular pressure in the renal artery clamp model and possibly other experimental forms of ARF.

3. Renal vasoconstriction

A reduction in renal blood flow (RBF) and a rise in renal resistance has been uniformly found in the initial phase of the glycerol model, renal artery clamping, and in the norepinephrine model. Ayer et al.(5), utilizing the xenon washout model, found a 19% decrease in RBF within 10 minutes after intramuscular glycerol administration in the rat, which progressed to a 73% fall at 24 hours. Similar figures have been reported with the hydrogen washout method(19), radioactive microspheres(50), and the electromagnetic flowmeter technique(64). Acute Ringer loading will reverse the vasoconstriction in the first 3 hours after glycerol administration with complete restoration of glomerular filtration rate (GFR)(64).

Arendshorst(2) recently evaluated the effect of 1 hour of renal artery occlusion on renal hemodynamics. Thirty to ninety minutes after release of the occlusion, RBF measured by electromagnetic flow transducer was reduced by approximately 40-50% and renal resistance was reciprocally increased. Similarly, Daugharty et al.(27) found approximately a 40% decrease in whole kidney and cortical single nephron filtration rate, after 3 hours of nearly complete occlusion of unilateral renal artery.

Fung(42) and subsequently Knapp and associates(55) first demonstrated that the prolonged infusion of large doses of norepinephrine would cause oliguric renal failure in the dog. When a dose of 0.75 ug/kg/min of the drug is given in the renal

artery, blood flow and urine flow fall to essentially zero and remain at this level for the length of the infusion. After discontinuation of the drug, there is a slow restoration of blood flow which rarely exceeds 50% of the control value at 3 hours(60). If the norepinephrine infusion is given for 2 hours, renal function is irreversibly damaged(23), although recovery will occur with a 40-min. infusion(24). However, in ARF caused by ischemia in rats(2), or by intrarenal norepinephrine in dogs(23), saline loading restores RBF to normal or even above normal <u>without</u> reversing oliguria. This suggests that reduced RBF per se does not account for the oliguria in the norepinephrine model. Nevertheless, a significant reduction in RBF is a well established phenomenon in the initial phase of ARF.

Within 24-48 hours after the initiation of ARF, alterations in RBF do <u>not</u> seem to play a major role in <u>maintaining</u> renal function impairment. In the glycerol model, the measurement of RBF has been variable in the maintenance phase of the condition. Chedru et al.(19) and Ayer & associates(5) have found a 70% decrease in RBF, Churchill et al.(20) found flow to be reduced by only 15%, and Hsu and colleagues(50)did not find any change at 24 hours in this model. Further, O'Connor et al.(64) demonstrated that Ringer loading could reverse renal vasoconstriction 18 hours after glycerol without restoring inulin clearance to normal. In contrast, there was a complete recovery of inulin clearance 3 hours after glycerol when volume expansion was utilized(64). Similarly, as mentioned already, Ringer loading can also restore RBF in both the renal artery clamping and norepinephrine model 24-48 hours after the insult without a

significant return of GFR.

The findings in the initial phase of nephrotoxic ARF are less uniform. Flamenbaum et al.(38) and Stein & associates(86) have both shown in studies in the dog an initial reduction in RBF of 50-60% in the low-dose uranyl nitrate model. In contrast, Blantz(11) utilized even higher doses of the nephrotoxic agent in the rat and found no change in RBF. Further, Mauk and associates(60) have administered a large dose of prostaglandin E_2 (a vasodilator) in the renal artery of the dog during uranyl nitrate administration and RBF was maintained 50% above the control level in 3 hours period after uranyl nitrate administration. A marked reduction in inulin clearance still occurred which was equivalent to the fall in this parameter in the nonvasodilated contralateral kidney. Thus in spite of an increase in RBF, a marked fall in inulin clearance still occurred in this model. In the dog, blood flow may be reduced in the initial phase of mercuric-chloride induced ARF(6). In any case, it seems likely from these data that renal ischemia is not always necessary for the development of ARF in these nephrotoxic models.

In addition, RBF has usually been found to be normal or even elevated within 48 hours after administration of these agents. Stein et al.(86) found this to be the case in the uranyl nitrate treated dog, whereas no change(51) or a modest increase(23) has been found in the rat after mercuric chloride. Baehler and associates(6) noted a marked increase in blood flow after Ringer loading in the dog, but oliguria persisted in mercuric chloride treated dogs. These studies, therefore, would seem to suggest that a reduction in RBF plays little if any in maintaining ARF in these

hemodynamically mediated and nephrotoxic models of ARF.

The precise intrarenal alterations in renal resistance in ARF have been a subject of controversy. Some investigators have felt that the fall in GFR and urine flow in ARF was disproportionately great for the degree of reduction of RBF and that this finding might be due to a combination of marked afferent arteriolar vasoconstriction coupled with efferent arteriolar vasodilation(7,76). In line with this view, the filtration filtration has been found to be reduced in experimental ARF as well as in studies in man(7,76). Yet it should be noted that a similar change would occur if tubular obstruction or a fall in the ultrafiltration coefficient (K_f) was operative in a given model of ARF. There is a paucity of direct measurement of afferent and efferent resistance in the various experimental models of ARF. In this respect, Daugharty et al.(27) studied glomerular dynamics after 3 hours of partial renal artery occlusion. Single nephron GFR and plasma flow were reduced proportionately, on average, by about 40%, in association with a proportional rise in afferent and efferent arteriolar resistance. Although it has been suggested that this model is not truly akin to clinical ARF (65), the fact remains that there are all graduations of renal impairment in the clinical setting(1). This particular model did have the advantage in that the renal cortical surface was homogenous and nephron GFR was readily and reproducibly measurable. In any case, further studies are warranted to directly measure glomerular dynamics in models of ARF.

The precise pathophysiologic basis for the increase in

renal resistance in various forms of ARF is still unclear. One group proposed that endothelial cell swelling was a self-perpetuating process induced by renal ischemia and maintained by the loss of the ability to regulate cell volume(39). More recently, however, this same group rejected this view because of the rapid return of blood flow in the particular model of ARF under evaluation(40).

There has been continuing interest in the possible role of the renin-angiotensin system in the regulation of renal resistance in ARF. The next section will further examine this controversial area. In addition, a brief discussion of the role of renal prostaglandins in the maintenance of renal resistance in these disorders seems in order.

4. Renin-Angiotensin system and ARF

Over 30 years ago Goormaghtigh(43) first noted changes in the juxtaglomerular apparatus of patients with ARF following crush injury. From these histologic changes, he suggested that the renin-angiotensin system may be involved in the pathogenesis of ARF. Subsequent work by other investigators produced further support for this postulate. The demonstration of increased plasma renin activity (PRA) in the initial stages of both clinical and experimental forms of ARF is also in line with this view(29,56,92). Although these observations are consistent with the possibility that the renin-angiotensin system is involved in ARF, they do not establish an etiological connection between activation of the renin-angiotensin system and the subsequent development of ARF. Further, it has been shown that PRA may return to control levels even though ARF persists. In addition, there are patients known to have marked elevations of PRA without the occurrence of ARF.

Patients with Bartter's syndrome, chronic vomiters, and cathartic and diuretic abusers, as well as many patients with congestive heart failure, cirrhosis and ascites, and the nephrotic syndrome, meet these criteria. The primary support for this thesis, however, stems from the experimental studies of various models, primarily in the rat, in which long-term salt loading protects against the development of ARF. This has been noted in glycerol-induced myohemoglobinuria(58), mercury(30) and dichromate(29) poisoning. In all these models, chronic salt loading almost completely protected against the development of renal ischemia and filtration failure seen in control water drinking rats. McDonald et al. (58) evaluated the course of acute renal failure after long-term dietary salt loading in rats. Ichikawa and his associates found that neither the administration of an angiotensin II inhibitor saralasin or a converting enzyme inhibitor SQ20,881 protected against the development of ARF(53). Although these drugs were able to raise RBF 3 hours after glycerol administration, neither agent reduced the level of BUN. Oken et al.(68) have also demonstrated that neither active nor passive immunization to angiotensin II significantly affects the degree of azotemia or the reduction in inulin clearance seen in animals with glycerol-induced ARF. These studies indicate that circulating angiotensin plays no direct and important role in the pathogenesis of this form of ARF. Although these studies do <u>not</u> exclude the possibility that renin release in ARF may have a major effect on the renal vasculature at the local site of release independent of circulating angiotensin activity, these findings certainly do not strengthen the case for this proposed mechanism.

5. Renal Prostaglandins in ARF

There has been a proliferation of data over the last decade concerning the role of prostaglandins in the regulation of renal hemodynamics, sodium and water balance. There is now unequivocal evidence that the primary renal prostaglandin, PGE_2, is a vasodilator, and it seems possible that alterations in the synthesis and release of this locally produced substance may play a role in the hemodynamic alterations which occur in ARF. Of possible interest in this regard is a recent study by Torres et al.(91) which demonstrated that indomethacin, an inhibitor of prostaglandin synthesis, increased the incidence and severity of ARF induced by glycerol. Since indomethacin alone has been demonstrated to decrease RBF, it may be that this alteration was mediated through an increase in renal resistance prior to the induction of ARF. This phenomenon may be of greater importance in models of ARF initiated by renal ischemia since indomethacin had no effect in mercuric chloride-induced renal failure. It does not seem likely, however, that a deficiency of renal prostaglandin production per se was operative in the glycerol model, since this same group of investigators found an increase in medullary PGE_2 concentration in the glycerol-treated rabbit. Further studies are needed to evaluate the role of renal prostaglandins in the pathogenesis of ARF.

6. Decrease in Glomerular Ultrafiltration Coefficient

A number of recent studies have focused on the change in ultrafiltration coefficient (K_f) in ARF. In two recent studies, a fall in K_f has been directly documented in experimental models of ARF. Blantz(11), studying the uranyl nitrate model in the rat, found that K_f decreased from a control value of 0.089 to 0.047

and 0.024 ml/sec/gram kidney wt./mm Hg 2 hours after administration of 15 and 25 mg/kg, respectively, of the drug. A similar reduction in K_f has been noted after gentamicin administration by Baylis et al.(9). More indirect evidence for a reduction in K_f has been noted in the norepinephrine and mercuric chloride models. Cox and associates(23) studied dogs with unilateral ARF induced by intrarenal norepinephrine 48 hours after the insult. RBF was reduced, but rapidly rose to a level even greater than that found prior to norepinephrine when the animal was volume expanded with Ringer solution. However, there was still no urine flow from the norepinephrine infused kidney. Examination of the surface tubules revealed they were all collapsed, a finding that would seem to be indicative of a primary failure of filtrate formation. In considering the possibilities that would explain the cessation of filtration in the presence of an increase in total RBF, an alteration in K_f seemed most likely. In order to further evaluate this possibility, transmission and scanning electron microscopy were performed. With transmission electron microscopy there was loss of the normal foot process (podocyte) structure with marked fusion detectable in virtually every glomerulus. This was even more dramatically demonstrated in the scanning studies. There was marked disruption of the normal podocyte structure. The cell bodies were unstructured and wrinkled, and it was not possible to differentiate the primary and secondary processes as well as the terminal foot processes. Similarly, study by Stein et al.(86) has shown similar but much less severe alterations in epithelial structures 48 hours after uranyl nitrate. It may also be of

interest that the total loss of normal epithelial structure seen with norepinephrine was associated with cessation of filtration while a less severe pathologic change occurred in the uranyl nitrate model in which nephron GFR was reduced by only 30%. Recently, Baehler and associates(6) evaluated the mercuric chloride treated dog and found functional changes quite similar to those in the norepinephrine model.

At present, there is little definitive information on whether the change in K_f in any of these circumstances is primarily due to alterations in effective capillary surface area or hydraulic conductivity. In circumstances associated with vasoconstriction, it is easy to visualize how K_f may fall. In this regard, Hornych and Richet(49) have shown that angiotensin II causes marked constriction and presumably a decrease in surface area of the glomerular capillaries. However, K_f has also been shown to fall in circumstances in which glomerular plasma flow remains constant or even rises(10,9,52). Although one might construe ways in which the effective surface area for filtration could still fall in this setting, it seems more likely that a decrease in capillary permeability would be responsible for the fall in K_f in this setting.

7. Biochemical Aspects of ARF

The pathophysiology which occurs after an ischemic or nephrotoxic insult are due to biochemical alterations at the molecular level. The sequence of events between the initial biochemical aberration and the observed renal insufficiency are undoubtedly complicated, and data on the biochemical basis of any particular model of ARF are scarce. Yet, there is apparently a

fundamental difference in the sequence of events which lead to these abnormalities in these two types of models of ARF.

An inadequate supply of oxygen and metabolic substrates to renal tissue is the initial, major biochemical aberration in ischemic ARF. The proximal tubule is largely dependent on oxidative metabolism for generation of adenosine triphosphate(ATP), which is required for metabolic transformations such as lipogenesis, protein synthesis, and maintenance of cellular structure. Distal portions of the nephron are able to synthesize ATP under conditions of limited oxygen availability(44). Therefore, energy dependent functions of the proximal tubule will be much more susceptible to hypoxia than those of the distal nephron. The number of anhydride-bound phosphate groups per adenosine moiety, called the energy charge, is the critical indicator of the state of the cell rather than ATP alone(4). Ischemic ARF probably decreases the energy charge such that cellular homeostasis is lost. A loss of cell viability and eventual necrosis will accompany a large decrease in energy charge. Reversal of the initiation of ischemic ARF will only occur if the energy charge remains above the level necessary for cell viability.

An initial decrease in RBF is <u>not</u> necessary for the development of renal functional impairment in nephrotoxic ARF, as noted previously. Rather than a generalized phenomenon such as a decrease in energy metabolism, a specific cellular insult related to the properties of the particular nephrotoxin may also be operative in this setting. Consider the model of mercuric chloride induced ARF.

Mercurial compounds react readily with sulfhydryl groups on

proteins, inhibit the activity of many enzymes, and affect numerous cellular processes(93). Organic mercurials have been shown to alter the transport of dyes and calcium in the flounder renal tubule(81). In mercuric chloride induced ARF(94) in rats, active transport of sodium and potassium decreased and an increase in passive leak occurred. It was suggested that mercaptide formation between the mercurials and sulfhydryl groups of plasma membrane proteins altered the permeability of the cell. Recent reports describing the effect of dithiothreitol (DTT) on mercuric chloride induced ARF supports the hypothesis that mercaptide formation with protein sulfhydryl groups is an initial step in the biochemical mechanism. DTT is a disulfide reducing agent(21) and a chelator of metals(22). If DTT is administered 30 min. after mercuric chloride injection into rats, the changes in parameters of renal function normally associated with ARF are negligible(82). DTT also reversed the inhibitory effect of mercuric chloride on sodium transport in the toad urinary bladder(82).

A direct interaction between uranyl nitrate (UN) and cell membrane components is also probably the initial step in this model of ARF. However, the interaction between uranium salts and cellular components is less well characterized than in the case of mercuric chloride, especially for mammalian cells. Yeast has been used to characterize the interaction and effects of UN on intact cells(79,80). UN does not readily penetrate the cell membrane, but reversibly complexes with phosphoryl and carboxyl moieties on the external surface of the membrane.

8. Synthesis of Experimental Data

Table 1 summarizes the various factors that are operative

in the few experimental models under discussion.

Table 1 Summary of pathogenetic factors operative in experimental ARF

Model	Renal Blood Flow		Tubular Obstruction	Filtration Leakage	Decreased K_F
	Initial	Maintenance			
Glycerol	↓	Ⓓ	±	?	?
Renal artery clamping	↓	Ⓓ	+	+	-*
Norepinephrine	↓	Ⓓ	+	?	+#
Uranyl nitrate	↓N↑	N	-	+	+‡
Mercuric chloride	↓	N↑	+	+	+#

Symbols: ↑, increased blood flow; N, normal blood flow; ↓, decreased blood flow; Ⓓ, models in which blood flow is reduced but can be markedly increased without restoration of inulin clearance; +, mechanism operative in a given model; ?, mechanism not evaluated adequately; -, mechanism not operative in a given model; *, only evaluated with incomplete clamping; #, indirect evidence for a fall in K_F; ‡, direct evidence for a fall in K_F.

Several comments should be made concerning this summary. As noted earlier, blood flow is reduced in both the initial and maintenance phase in all three hemodynamic models. However, a restoration of RBF with Ringer solution in the period of fixed renal failure does not cause a significant rise in GFR as measured by inulin clearance. In the nephrotoxic models, blood flow is more variable and there seems to be definite situations in which renal ischemia is not necessary for the development of ARF(60). Thus, renal ischemia does seem to be a common denominator in the hemodynamic but not in the nephrotoxic models. In addition, other factors must be involved in the maintenance of ARF in all of these experimental maneuvers. In the last three columns we have attempted to delineate what these factors may be. Surprisingly,

the glycerol model seems the most confusing. Although it has been assumed by others that renal vasoconstriction is also important in the maintenance period of glycerol-induced ARF(66), O'Connor and associates(64) have shown that Ringer loading will restore blood flow without return of GFR 18 hours after glycerol. Although incomplete tubular obstruction in the distal nephron may also be present, this does not seem to be the total explanation for the functional renal impairment. In the remaining models, two or all three of the other identified pathogenetic factors seem to be operative in some stage of ARF.

With these data in mind, Fig. 1 summarizes the current view of the pathogenesis of ARF. The initiating event is either renal ischemia or the administration of a nephrotoxic agent. Although this later intervention may cause renal functional impairment without a change in blood flow, hemodynamic alterations may also occur. The eventual alteration in renal function will then be determined by the severity and specific type of renal insult and the various poorly characterized modifying factors which may either increase or decrease the sensitivity of the nephron to the particular insult. This will lead to a variety of biochemical alterations in cellular function and integrity which will be expressed by the various maintenance factors designated in the figure.

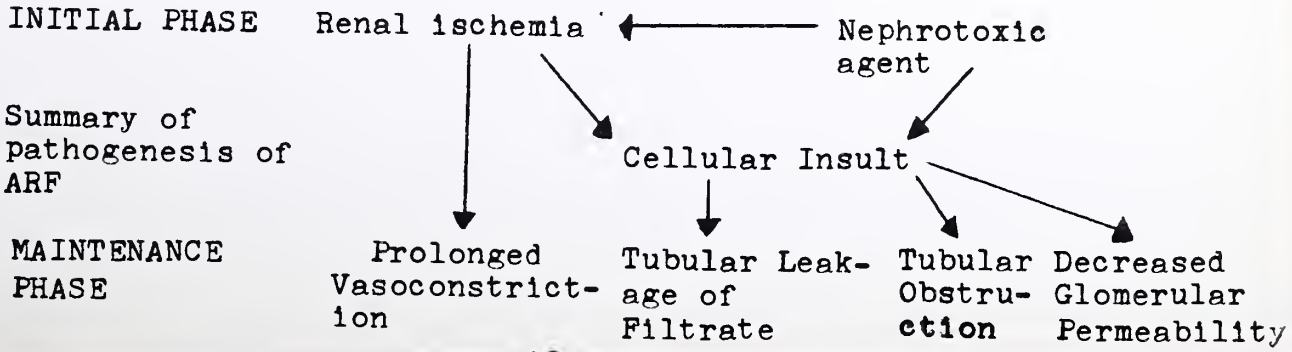


Fig.1 Summary of pathogenesis of ARF

Last of all, a comment should be made concerning the similarity of these models to the clinical setting. Although it is obviously difficult to extrapolate the findings in these models to clinical ARF, we feel that several of these models are similar in a number of respects. For instance, the glycerol model is, in a number of respects, analogous to the "crush syndrome" seen after trauma of various types in man. Further, it should be recognized that clinical ARF may encompass a wide range of renal functional impairment. Thus, the partial renal artery clamping model described by Daugharty et al.(27) may indeed be analogous to a less severe form of ARF in which reversal of the functional impairment is prompt. In any case, I feel that these models are powerful tools for the study of ARF and that their use has greatly increased our knowledge in this field.

B. Observations in man

A knowledge of certain aspects of the pathology of acute renal failure (ARF)(46) in man is vital for an understanding of pathogenesis. The histologic picture differs greatly between post-ischemic failure and nephrotoxic failure. In nephrotoxic injury, damage to tubular epithelium is prominent and relatively uniform, especially in the proximal convolutions. In post-ischemic failure, epithelial-cell damage is most often focal or patchy. When present, necrosis may occur in any part of the tubule, but it is most frequent in distal tubular segments, especially in the corticomedullary junction. Proximal tubules are often dilated and lined with flattened epithelium, but necrosis is uncommon.

From the pathogenetic point of view, the most striking feature of post-ischemic ARF is the poor correlation between histologic changes and functional severity. The focal and often minimal damage to tubular cells contrasts to the severe impairment of renal function. For example, Finkh et al.(35) compared coded specimens from patients with clinically definite ARF with specimens from control patients who had died without any evidence of impaired renal function. Histologic evidence of tubular damage was often slight and occasionally absent in the specimens from patients with renal failure. Similar histologic changes were occasionally present in the control specimens. The histologic and clinical features did not show the degree of correlation to be expected if tubular cell necrosis were a critical part of the mechanism of renal failure. Such observations have led many investigators to believe that functional changes such as vasoconstriction are the principal factors. However, subtle damage to intracellular organelles may be noted on electron microscopy(26) in cells that

are not overtly necrotic, and biochemical damage may be even more widespread. Thus, although histologic observations make it unlikely that necrosis per se is the key mechanism, they do not rule out functionally important cell damage. Routine histologic studies of tubules in the cortex do not suggest widespread tubular obstruction by intraluminal debris. However, limited observations (72) suggest that casts, possibly composed in part of Tamm-Horsfall mucoprotein, may be more prominent in the medullary collecting ducts. (Tamm-Horsfall mucoprotein is a component of normal urine that is readily precipitable in vitro under conditions of flow and urinary composition that might be expected in the collecting ducts of hydropenic subjects(72).) The dilatation of proximal tubules described above may also be attributable to obstruction in more distal segments. With reference to reduction of renal blood flow or glomerular filtration as primary pathophysiological mechanisms, it is noteworthy that the blood vessels and glomeruli appear normal on light and electron microscopy(26,71,25) in human ARF.

The pathological features of post-ischemic ARF in man cast some doubt on the relevance to human disease of the experimental models that have been studied most carefully. Models in which nephrotoxins such as mercury or dichromate are used to induce ARF presumably are pertinent to nephrotoxic failure in man but are probably less valid for predicting mechanisms in human post-ischemic failure. Complete occlusion of the renal artery has been studied extensively as a model of the latter. However, cell damage is much more severe and diffuse after arterial occlusion in rats(31,2) than in typical post-ischemic ARF in man

(46,33). Other ischemic models(23) induce glomerular changes not found in specimens from patients with acute renal failure. These differences in no way deny the value of careful studies of experimental models. They do suggest the importance of validating experimental hypotheses derived from animals by studies of patients whenever possible, despite the limitations imposed by ethical and practical considerations.

Renal vasoconstriction persists throughout the period of renal failure in patients with ARF. Total renal blood flow (RBF) is usually between $\frac{1}{4}$-$\frac{1}{2}$ of normal, as estimated by a variety of methods, such as dye dilution and inert-gas washout(63,47). On angiography, the cortical arterial vessels are narrowed or not visualized, and the cortical nephrogram is absent. The fast, "outer cortical" component of inert-gas washout curves disappears or is markedly slowed(47). These changes have been observed both in ischemic and in nephrotoxic ARF(48). From these studies, Hollenberg and his associates(47) have inferred that severe, sustained preglomerular vasoconstriction is the pathogenetic final common pathway in all forms of human ARF. Observations of Reubi et al. in patients with post-ischemic ARF led them to a similar conclusion(75). These investigators used a multiple indicator-dilution technic to estimate the volumes of the vascular, interstitial and intratubular components of the kidney. They reasoned that, if glomerular filtration were abolished, the distribution volume of their marker of tubular + interstitial/vascular (^{24}Na) would be decreased, because the ^{24}Na would be denied access to tubular lumens. On the contrary, if glomerular filtration were

preserved but tubular cells had become leaky to filtrate and large molecules, the distribution volume of their index of vascular/interstitial volume (^{51}Cr-EDTA) would increase and approach that of ^{24}Na. Their results were consistent with the hypothesis that glomerular filtration is suppressed. As mentioned earlier, RBF during the oliguric phase was about ½ normal. Thus, the suppressed filtration could, at least in part, be due to afferent arteriolar vasoconstriction. Plasma renin activity (PRA) is increased in most patients with ARF during the period of renal insufficiency and returns to normal as renal function improves(14). These data fit with the hypothesis that angiotensin II is the cause of persistent vasoconstriction, although they obviously do not separate cause and effect.

Some observations in patients do not fit very well with the concept that renal vasoconstriction and suppressed filtration are the principal causes of renal failure. During infusion urography, a nephrogram appears promptly and is of normal intensity in most patients with ARF(16). Iodinated contrast mediums are excreted by filtration and are not reabsorbed; the nephrogram is due to collection of the contrast material in proximal convolutions(41). Hence, as pointed out by Fry and Cattell(16,41) the early and intense nephrogram suggests that glomerular filtration is <u>not</u> greatly suppressed in patients with ARF. Moreover, the nephrogram usually persists unchanged for hours or even days. In oliguric patients, little or no contrast is being excreted, and a pyelogram does not develop. Thus contrast is entering the tubules by filtration but not leaving in the urine, yet the nephrogram usually does <u>not</u> become increasingly dense. To account for this

finding, Fry and Cattell suggest that contrast material may be leaking back through damaged tubular epithelium. Although these observations are of considerable interest, it is well to remember that the intensity of a nephrogram can only be estimated semiquantitatively. From these data, therefore, one can only infer with confidence that filtration is occurring at a substantial rate, not that the filtration rate is normal. Moreover, studies in rats with experimental ARF show the same radiologic phenomena, and yet analysis of renal failure demonstrates that the amount of contrast medium in the kidney is substantially less than in controls(78). This finding indicates that a normal appearing nephrogram is not proof of a normal rate of filtration of contrast medium. Despite these caveats, radiologic observations do not fit well with the hypothesis that vasoconstriction and suppressed filtration are the key factors causing oliguria in human ARF. Another type of observation also poses problems for this hypothesis. Infusion of vasodilators such as hydralazine, acetycholine(57) and prostaglandins(74) into the renal artery markedly increases renal blood flow in patients with ARF, and yet oliguria persists. These observations are similar to those in a number of experimental models, as described earlier. One can explain them by postulating a combination of afferent arteriolar vasoconstriction and efferent arteriolar dilatation in ARF. In that event, vasodilators might restore blood flow by efferent dilatation without restoring glomerular filtration pressure to normal(75). There is no direct evidence for this hypothesis, however; it is merely an ad hoc assumption to avoid the necessity of discarding vasoconstriction as the key pathogenetic mechanism in human ARF.

C. Purpose of the thesis

In the foregoing discussion of experimental ARF, the pathophysiological event of backleak of glomerular filtrate across damaged tubular epithelium has been convincingly demonstrated using micropuncture techniques in a number of animal models. Both ischemic or nephrotoxic insults appeared to have the potential of establishing this kind of tubular dysfunction. Whether a similar mechanism operates in human ARF is still unknown. To test this hypothesis in human ARF, we tried to determine the fractional clearances (sieving coefficients) profile of dextran in patients with hemodynamically mediated ARF. Dextran-40 (Rheomacrodex®) is a homologous series of neutral macrosolutes-dextrans- of varying molecular ~~molecular~~ weight and size (average molecular weight $\overline{M.W.}$=40,000; Einstein-Stokes radius (ESR) from 20-57 Å). Like inulin, dextran has the properties of neither secreted nor reabsorbed by the normal tubule, so that its fractional clearance (≡clearance of dextran/clearance of inulin) can be equated with the concentration ratio of dextran in Bowman's space to plasma (BS_{D_i}/P_{D_i}). The concentration of a particular dextran species D_i of ESR r_i in Bowman's space would be expected to vary inversely with ESR, larger molecules being more restricted from the Bowman's space because of the size-permselectivity properties of the glomerular barrier(17,18). The other important determinant of the fractional clearance of a macrosolute is the single nephron glomerular filtration rate (sngfr), the rate itself being determined by several physiological variables as well as the permeability properties of the glomerular membrane. Several studies have shown that the Bowman's space-to-plasma concentration ratio of a macromolecule should, in general,

depend inversely on sngfr(18). The effects of individual determinants of sngfr on fractional clearances of dextran have been well studied in the normal Munich-Wistar rat(13,17,18). These determinants are: (1) mean transcapillary hydraulic pressure difference, $\overline{\Delta P}$; (2) initial glomerular plasma flow rate, Q_A; (3) systemic (afferent arteriolar) plasma protein concentration, C_{PA}; and (4) glomerular capillary ultrafiltration coefficient, K_F. If it is assumed that in ARF the transglomerular permeation of dextrans is the same as that of the normal controls (the validity of this assumption and the reasoning behind it will be discussed in Methods and Discussion), i.e. the fractional clearances of dextrans as would be determined from the fluid in the Bowman's space of the ARF patients (if indeed we could sample the glomerular filtrate under those circumstances) should be the same as that of controls. That being the case, no perturbation of transport dynamics could possibly account for values of fractional clearances of dextran (θ_{D_i}) above unity, unless the observed urinary inulin clearances in these ARF patients as determined by the standard method $C_{IN}=\dot{V}_u \times U_{IN}/P_{IN}$ is less than the true inulin clearance (i.e. GFR), a situation that could arise if there is tubular damage in ARF permitting backleak of inulin. Based on this assumption, and relating the observed fractional clearances profile of dextrans ("apparent" sieving coefficients) obtained from the ARF patients with that of normal controls, one could estimate the amount of backleak of inulin in the damaged tubules in human ARF using mass conservation mathematical models. This thesis attempts to provide an answer to that issue.

II. Methods

Rationale. In the normal kidney, inulin and uncharged dextran molecules with Einstein-Stokes radii (r) up to 44 A are excreted by glomerular filtration, and neither secreted nor reabsorbed (17,18). While inulin (ESR=13.5 Å) is freely filtered, restriction to filtration is evident for dextran molecules of ESR=22 A and greater. The glomerular fractional clearance or sieving coefficient (θ_D) for a dextran molecule (D) of a specific size i can therefore be determined from its fractional urinary clearance relative to inulin(13). Employing polydisperse dextran 40 (Rheomacrodex®, Pharmacia Fine Chemicals, Uppsala, Sweden, ESR=20-57 Å), we determined the fractional dextran clearance profile (or glomerular sieving curve) in 7 healthy volunteers. As seen in Figure 1, when fractional clearance values for narrow dextran fractions in these volunteers are plotted as a function of ESR (r) of the dextran, measurable restriction to dextran (θ_D<1.0) increases with molecular size from r=22 to 40 Å.

Glomerular permselectivity to dextran molecules in ARF might differ from that in healthy subjects due to altered glomerular capillary hemodynamics and a reduction in the glomerular ultrafiltration coefficient(13,13A,17). No alteration in glomerular transport dynamics could, however, possibly account for values of

above unity, a finding which we observed for lower molecular weight dextrans (r=22-28 Å) in 7 of 10 consecutively examined patients with ARF. This finding can only be reconciled if the urinary inulin clearance is less than the true glomerular filtration rate (GFR). This situation could arise if tubular damage permitted tubular backleak of inulin into the peritubular space. If it is assumed that in ARF either the permeability properties of the glomerular

capillary wall are identical to those of the normal glomerulus, or any increase in transglomerular permeation of macromolecules due to reduced plasma flow(12,17) is compensated by a decrease in the glomerular ultrafiltration coefficient(17), then the normal dextran sieving curve may be used for estimating "true" sieving coefficients of filtered dextrans in Bowman's space in ARF. Based on this assumption and employing the measured "apparent" sieving curve obtained in ARF and mass-conservation constraints, we estimated the backleak of inulin from damaged tubules in human ARF.

Patient Population. Ten consecutive patients developing ARF following open heart surgery and 2 groups of control subjects were studied using a protocol and consent procedure that had been approved by the Committee on the Use of Human Subjects in Research at Stanford University. This discrete variety of ARF was chosen because it is hemodynamically mediated and usually accompanied by a high rate of urine flow. ARF, identified by the sudden onset of azotemia, became manifest within 24-48 hours of surgery in 7 patients. In these patients the ischemic insult was thought to have been delivered during the surgical procedure, the magnitude and difficulty of which was reflected by a nearly two-fold prolongation of cardio-pulmonary bypass time (160±29 min. (mean ± standard error)) above the institutional average. In the remaining 3 patients ARF developed between the 3rd and 5th post-operative days in the wake of a prolonged low cardiac output syndrome and superimposed discrete hypotensive episodes. Clearance of macromolecules (dextrans) was performed when ARF had been established for at least 48 hours and prior to initiation of dialysys therapy or resolution. During this period, average

serum creatinine concentration rose from 1.5±0.01 to 4.5±0.4 mg/100 ml, while the corresponding values for blood urea nitrogen were 24±3 and 88±6 mg/100 ml, respectively.

Seven healthy male and female volunteers aged between 20 and 65 years comprised a normal control group. Twelve patients with a maximum postoperative serum creatinine <2 mg % following open heart surgery served as cardiac surgical controls. Age (44 to 77 years), sex distribution (9 male, 3 female), and cardiac performance (average cardiac index=2.64±0.13 L/min/M^2) were similar to those in the ARF population (Table 1). As a consequence of transient postoperative reduction of cardiac performance, GFR was depressed relative to the normal control group (76±9 vs. 102±5 ml/min/1.73 M^2). As can be seen in Figure 1, there was a modest elevation of fractional dextran clearance for the dextran r range 24-40 Å for this group relative to normal controls ($P<0.05$). This increase in transglomerular passage of dextrans is probably attributable to depressed renal plasma flow rather than to changes in the permeability properties of the glomerular capillary wall(12).

Study Protocol. All studies were performed when the patients were hemodynamically stable. Sudden changes in blood volume, viscosity and oncotic pressure were avoided by withholding infusion of packed red cells, hyperoncotic human albumin solution and furosemide for at least 6 hours prior to study when clinically feasible. During each study, fluid balance was maintained by constant infusion of an isotonic dextrose solution. Radial arterial pressure was monitored throughout the study via a saline-filled catheter, which was connected to a Hewlett Packard pressure

transducer (Model HP 1280). Cardiac output was determined by injection of indocyanine green through a pulmonary arterial catheter with subsequent analysis by a Waters densitometer (Model D-400) and Waters Cardiac Output Computer (Model CO-4).

Inulin clearance was performed by administering a priming dose (24 mg/kg) followed by constant infusion which resulted in a steady state plasma inulin concentration of 10-15 mg/100 ml. Dextran 40 (130 mg/kg, concentration 10% solution) was administered by intravenous injection directly following the inulin prime. After an equilibration period lasting 60 minutes, three timed (20 min. each) urine collections were made and blood was drawn at the beginning and end of each collection. Inulin clearance was calculated as

$$\text{clearance}_{IN} = (U/P)_{IN} \cdot \dot{V} \qquad (1)$$

where $(U/P)_{IN}$ is the <u>average</u> urine-to-plasma inulin concentration ratio and $\dot{V}$ the average urine flow rate in ml/min. for the three collection periods.

Fractional clearance of dextrans (θ_D) was determined for the first timed collection period as

$$\theta_D = \left[(U/P)_D/(U/P)_{IN}\right] \qquad (2)$$

where $(U/P)_{IN}$ is the urine-to-plasma dextran concentration ratio.

<u>Determination of Fractional Inulin Leakage</u>. When renal function is normal, all inulin and dextran cleared by the glomerulus appearing in the glomerular filtrate is assumed to appear in the urine. It is this assumption which permits estimation of the sieving curve for dextran ultrafiltration by the glomerular capillary wall(13). For the kidney in ARF, however, we propose that tubular backleak of inulin (and also inulin) occurs; if we

further assume that such leakage is "passive" (that is, occurs solely by ultrafiltration and/or diffusion through the damaged tubular epithelium), then the rate of leakage of any such solute should be nearly proportional to the concentration of that solute in the glomerular filtrate. Under such circumstances, the fractional backleak of inulin (defined as the rate of backleak of inulin divided by the glomerular filtration rate) will be constant (k_{IN}) and independent of the inulin concentration in the plasma. Precisely the same criteria apply to any dextran species of molecular size (i), leading to a fractional dextran backleak of k_{D_i}. However, the permeability of the damaged tubular wall to dextrans would be expected to be a declining function of the size of the dextran molecule:

$$k_{D_i} = f(r_{D_i}) \qquad (3)$$

where $k_{D_i} \dashrightarrow k_{IN}$ as $r_{D_i} \dashrightarrow r_{IN}$; $k_{D_i} \dashrightarrow 0$ as $r_{D_i} \dashrightarrow \infty$.
Simple mass-balance computations on inulin and dextran cleared by the glomerulus (see Appendix for details) lead to the following relationship:

$$\theta_{app} = \theta\left[(1-k_{D_i})/(1-k_{IN})\right]$$
$$= \theta\left[(1-f(r_{D_i})/1^{-}k_{IN})\right] \qquad (4)$$

where θ_{app} is the "apparent" sieving coefficient for the ith dextran based solely on plasma and urine concentration measurements, and θ is the "true" sieving coefficient of the glomerular capillary wall for that dextran. Qualitatively, we can deduce from equation (4) that, for large dextran molecules where $k_{D_i} \simeq 0$, $\theta_{app} > \theta$ (since $(1-k_{IN})$ 0), while for small dextran molecules where $k_{D_i} \simeq k_{IN}$, $\theta_{app} \dashrightarrow \theta$.

Assuming that the dextran sieving curve in Bowman's space in ARF is identical with that in normal controls for the reasons

given above, equation (4) can be reduced to:

$$\theta_{app}/\theta = (1-k_{D_i})/(1-k_{IN})$$
$$= \left[1/(1-k_{IN})\right] - \left[k_{D_i}/(1-k_{IN})\right] \qquad (5)$$

Now, k_{IN} is a constant for a given subject, whereas k_{D_i} is a function of r_{D_i}. As $r_{D_i} \rightarrow r_{IN}$, $k_{D_i} \rightarrow k_{IN}$; and as $r_{D_i} \rightarrow \infty$, $k_{D_i} \rightarrow 0$. Hence, the ratio (θ_{app}/θ) should increase with r_{D_i} from a value of unity (for small r_{D_i}) to a limiting value of $1/(1-k_{IN})$ as r_{D_i} increases. Since, as $r_{D_i} \rightarrow \infty$, $\theta \rightarrow 0$, a plot of (θ_{app}/θ) vs should permit a reasonably accurate extrapolation to the limiting value of $(\theta_{app}/\theta) \rightarrow 1/(1-k_{IN})$. Inasmuch as the "true" GFR is simply equal to $1/(1-k_{IN})$ times the "apparent" GFR, the glomerular filtration rate corrected for tubular backleak of inulin can be calculated.

Laboratory Determinations.

Inulin clearance determination. For the calculation of inulin clearance, inulin concentrations in plasma and urine were determined using the autoanalyzer method of Fjelbo and Stamey(36). Figure 4 in the Appendix illustrates the flow diagram of the set-up. The method uses the following reagents:

a. HCL-Ferric Chloride: 7.5 mg $FeCl_3$ in 1000 ml concentrated HCl.
b. Resorcinol (Sigma Chemicals Co.): 1.5 gm resorcinol up to 1000 ml with 95% ethanol.
c. Brij-35 in water to keep the bubble pattern even.

The reagents were mixed in two long mixing coils before sending them to a 80 C^{o} heating bath. It circulated in the bath for ~10 minutes where inulin, a polymer of fructose, was hydrolyzed by the HCl to fructose, which, on reaction with resorcinol, produced a yellow color. The yellow color was measured at 480 mμ in the

colorimeter. We have found this method to be uninfluenced by the presence of dextran.

Chromatographic Procedures. To determine fractional dextran clearance, plasma and urine samples from the first timed collection were submitted to gel-permeation chromatography on Sephacryl S-200 columns. The Dextran-40 (Rheomacrodex®) in plasma and urine was separated into narrow fractions (ESR of 2 Å range) on Sephacryl® S-200 Superfine gel medium (Pharmacia Fine Chemicals, Inc.), prepared by covalently cross-linking allyl dextran with N, N' methylene bisacrylamide. This new kind of gel filtration medium combines a highly porous gel structure with excellent chemical and physical stability. It was packed in three K 16/100 Pharmacia columns (Pharmacia Fine Chemicals, Uppsala, Sweden), each with the following column characteristics:

Internal Diameter (I.D.) = 1.6±0.002 cm

Outer Diameter (O.D.) = 1.925±0.035 cm

Length of Column (L) = 91, 95 and 91 cm respectively

Cross sectional area (A) = 2.0 cm^2

Maximum Volume (V_{MAX}) = 200 ml

Column flow rate (Q) = 35 ml/hour (17.5 ml/cm^2-hour)

With 0.3% saline as eluant (0.05% trichlorobutanol added to prevent microbial growth), 2.6-ml eluted fractions were collected with an automatic fractionator (Gilson). The void volume (V_o) for the individual columns were determined with blue dextran, and the results were as follows:

	Column A	Column B	Column C
V_t:	180 ml	185 ml	180 ml
V_o:	61 ml	65 ml	64 ml

where V_t is the total volume of the gel column and V_o the corresponding void volume, i.e. the volume between the swollen Sephacryl particles.

Calibration of Columns. Calibration curves of the partition coefficient (k_{av}) (or the fractional volume available to the solute) vs. molecular weight were determined using known molecular weights of Dextran T-fractions, namely T-10 (average molecular weight ($\overline{M.W.}$) =9,400, ESR (r)=22.8 Å), T-40 ($\overline{M.W.}$=41,000, r=45.1 Å) and T-70 ($\overline{M.W.}$=67,000, r=56.9 Å). A linear relationship was obtained between the partition coefficient k_{av} and $\log_{10}\overline{M.W.}$. The k_{av} of each narrow dextran fraction was calculated using the equation

$$k_{av} = (V_e - V_o)/(V_t - V_o) \qquad (6)$$

where V_e is the elution volume of the solute (dextran fraction).

Einstein-Stokes radii (r) for individual dextran fractions were calculated using the equation

$$r(\text{Å}) = 0.33.\overline{M.W.}^{0.463} \qquad (7)$$

where $\overline{M.W.}$ is the average molecular weight of that dextran fraction as determined from calibration curves (k_{av} vs. $\overline{M.W.}$) (unpublished data, personal communications with Pharmacia AB, equation (7) is valid for dextran of type B 512).

The calibration results for each column were tabulated below. Figures (1), (2), and (3) in the Appendix were the calibration curves respectively.

Column A:	M.W.	Elution volume (V_e), ml	k_{av}
	9,400	124.0	0.529
	41,000	87.9	0.226
	67,000	77.3	0.137

Column B:	M.W.	Elution volume (V_e), ml	k_{av}
	9,400	119.6	0.455
	41,000	85.8	0.173
	67,000	78.0	0.108

Column C:	M.W.	Elution volume (Ve), ml	k_{av}
	9,400	117.0	0.457
	41,000	85.8	0.188
	67,000	75.4	0.098

For separating the dextrans in the urine and plasma into narrow (2 Å) fractions, a 2-ml protein-free filtrate of the first timed urine collection and a 2-ml protein-free, equal volume mixture of the two plasma samples drawn at the beginning and end of that urine collection were applied to the same Sephacryl S-200 column consecutively. Protein-free filtrates of plasma were prepared by adding 1 ml of 10% $ZnSO_4$ solution, 1 ml of 0.75 N NaOH solution, 1 ml of plasma to 2 ml of 0.3% saline to obtain a 1:5 dilution, followed by centrifugation and decantation. Protein-free filtrates of urine were prepared in a similar manner, but were diluted in such a way as to bring the urinary dextrans concentrations into range with the dextran standards. This could be anywhere from a 1:5 to 1:200 dilution, depending on the urinary concentration of dextran. The first 31 eluted fractions (corresponding to the first 31x2.6 ml = 80.6 ml) coming of the chromatographic column were discarded, while fractions #32-61 were collected for dextrans and inulin analysis (see below). The partition coefficients (k_{av}) of the eluted fractions from each individual column were calculated using equation (6). The <u>average</u> molecular weight ($\overline{M.W.}$) of each fraction was then determined from the calibration curves of figures (1), (2), and (3) in the Appendix. The Einstein-Stokes radii (r) of each fraction could then be calculated using equation (7).

<u>Dextran and inulin concentrations analysis</u>. Dextran and inulin concentrations of the eluates (fractions #32-61 of both urine

and plasma) were determined using a modification of the Autoanalyzer anthrone method of Scott and Melvin(83). Figure (4) in the Appendix illustrates the flow diagram of the set-up. The reagents used had the following composition:

a. 1.0 gram of anthrone per liter of 75% (v/v) concentrated H_2SO_4 + 2.0 ml of Brij-35 per liter of solution

b. 3.0 ml Brij-35 per liter of deionized water.

The processing rate was 40 samples per hour with a 1:1 (30 sec) ratio for aspiration and washing time. After mixing with the anthrone and H_2SO_4, the sample was conveyed to a delay coil at 95°C where it circulated for 10 minutes during which time the dextran was hydrolyzed by the H_2SO_4 and the glucose produced reacted with anthrone to give a green color. After a short cooling in a mixing coil, the color was measured at 625 nm in a 15 mm flowcell of 0.05 volume. This method is best applicable for assaying dextran concentration in the range of 0-100 μg/ml, and for that reason the working standards of dextran therefore range from 1 to 80 μg/ml. Figure (5) illustrated the eluted fractions of a solution containing 100 mg% Dextran 40 and 50 mg% inulin coming off the chromatographic column and then subjected to the anthrone assay. First came the early dextran-containing fractions (#1-14), while the smaller inulin molecules (r=11-17 Å) appeared in late eluted fractions (#17-26). A separate set of inulin standards were used for assay of these inulin concentrations. It should be mentioned that chloride ions interfere with the anthrone method. As stated earlier, the eluted fractions of the plasma and urine all contained chloride because of the saline eluant. To compensate for this chloride interference, the dextran and inulin in the working standards were also

dissolved in 0.3% saline. In addition, since inulin is a polyfructose, it would also be hydrolysed by sulfuric acid, and the fructose produced also reacted with anthrone to give a similar colored compound like dextran. Hence, in order to avoid the interference from inulin, only the fractional clearances (sieving coefficients) of dextrans corresponding to ESR of 20 Å or greater would be presented.

Miscellaneous. Urine and plasma sodium concentrations were measured using standard flame photometry. Urine and plasma osmolalities were determined by the freezing-point depression method.

Statistical Methods. All results are expressed as the mean and standard error of the mean. Student's t test for unpaired data was used to evaluate differences between the experimental and control groups.

III. Results

The results of 10 studies during the course of established ARF are compared with those of 12 nonazotemic postoperative control patients in Table 1. The profound depression in renal function is indicated by the inulin clearance, which averaged 10 ± 2 ml/min/1.73 M^2, a value corresponding to only 13% of that in control patients (76 ± 10 ml/min/1.73 M^2). Only one patient was oliguric ($V = < 0.5$ ml/min); urine flow averaged 1.9 ± 0.4 ml/min in all 10 studies. Elevation of fractional sodium excretion relative to control patients (7.9 ± 2.1 vs. 1.4 ± 0.4%), isosthenuria ($U/P_{osmolality}$ 1.05 ± 0.03), and a low urine-to-plasma inulin concentration ratio (7 ± 1) are all consistent with the diagnosis of nonoliguric ARF(62). That this ARF was acutely unresponsive to correction of prerenal factors is suggested by persisting depression of inulin clearance at a time when cardiac index (2.40 ± 0.19 vs. 2.64 ± 0.13 liters.min^{-1}.m^{-2}) and mean arterial pressure (75 ± 3 vs. 81 ± 1 mm Hg) were not significantly different from corresponding values in control patients who had near normal renal function.

In Fig. 1 the mean measured fractional clearances of dextran for ARF subjects, normal subjects, and postcardiac surgical controls are plotted as a function of Einstein-Stokes radius, r_{D_i} interval 20-40 Å. As can be seen, the fractional clearance profile (θ_{app}) in ARF subjects was strikingly elevated above that of both control groups. When compared to normal subjects, elevation of θ_{app} was highly significant at the $P<0.005$ level for all dextran fractions $r_{D_i} > 22$ Å. The level of significance for θ_{app} vs. θ of cardiac surgical controls over the same range varied from $P<0.02$ to $P<0.001$. In contrast to both groups of controls in whom

restriction to dextrans with r = 22 Å or greater was evident, θ_{app} remained at or above unity for the dextran r interval 20-28 Å in ARF. Table I in the Appendix is a tabulation of the fractional dextran clearances from r = 20 to 40 Å in the patients with ARF.

A plot of the ratios of the average values of θ_{app} (ARF) to θ (normal controls) as a function of normal fractional clearances (θ), is shown in Fig. 2; the correlation is nearly linear in the interval $1.0>\theta>0.2$ and the asymptotic value of (θ_{app}/θ) is estimated to be 2.0. Accordingly, approximately 50% of the filtered load of inulin is lost via leakage from damaged tubules, and the true GFR for these patients is about twice that calculated by urinary inulin excretion. By plotting fractional dextran clearance profiles for normal controls and the ARF patients on <u>semilogarithmic</u> coordinates as a function of dextran r (Fig. 3), the relative changes in θ_{app} and θ with increasing values of r become much clearer. As Fig. 3 shows, the θ vs r_{D_i} curves become virtually straight lines for values of $r_{D_i}>32$ Å for both populations, and more importantly, parallel each other. This condition corresponds to a constant ratio (θ_{app}/θ) of about 2.0, which verifies the computation that about half the filtered inulin leaks out of the damaged tubules.

Since there was considerable variability between the apparent fractional dextran clearance profiles for the 10 ARF studies, the data for each study were plotted independently in the manner of Fig. 2; and the individual asymptotic values of (θ_{app}/θ) were computed. The results of these extrapolations are summarized in Table 2. The limiting values $1/(1-k_{IN})$ varied between 1.4 and 3.6, indicating that there may be considerable variability in the extent

of tubular damage, with the result that transtubular inulin backleak, while substantial in all cases, may differ by more than twofold among individuals (range from 31-72%). True rates of glomerular filtration calculated from the product of measured inulin clearance and $1/(1-k_{IN})$ varied from 6 to 33 ml/min in this variety of ARF (Table 2).

Reexamination of equations (A4) and (A5) (see Appendix) reveals that the quantities k_{D_i} and k_{IN} represent the rates of transport of the ith dextran and inulin across the damaged tubular epithelium when compared at equal solute concentrations in the glomerular filtrate. The ratio (k_{D_i}/k_{IN}), therefore, may be regarded as the "relative permeability" of the tubular epithelium to the ith dextran with respect to inulin. Since the precise transport mechanism across the tubular wall in ARF is unknown, this quantity cannot unambiguously be regarded as a "sieving coefficient" for the damaged tubule. Nevertheless, tubular morphology(32) and microinjection studies(88) in experimental ARF suggest that the transmural passage of inulin is unlikely to be restricted. We have, therefore, compared the variation of k_{D_i}/k_{IN} with r_{D_i} and with the sieving curve of the normal glomerulus. The results of such computations and comparisons are shown in Table 3 and Fig. 3. It would appear that the damaged tubule's relative permeability and the normal glomerular sieving curve are nearly identical for macromolecules of Einstein-Stokes radii $r<28$ Å, but the former declines much more rapidly than the latter for molecules in Einstein-Stokes radius range $30\ Å<r<40\ Å$. This observation supports the finding that intraluminal macromolecules of the size of albumin ($r = 36$ Å) are virtually unable to escape through the tubular wall in ARF(88).

TABLE I

CLINICAL AND LABORATORY FEATURES OF ARF IN 10 PATIENTS AT THE TIME OF FRACTIONAL DEXTRAN CLEARANCE

Patient	Age/Sex	Days of ARF§	CI* L/min/M²	MAP mm Hg	$\dot{V}$ ml/min	Clearance ml/min/ 1.73 M²	FE† %	U/P_{IN}♦	U/P_{osm}‡
1	75/M	5	2.48	72	2.7	11.7	17.4	4.2	1.12
2	60/M	3	3.07	67	3.1	13.3	12.3	4.4	0.93
3	77/M	3	-ND	84	3.8	14.5	15.6	4.3	1.02
4	77/F	3	1.71	74	0.8	10.4	2.4	11.0	1.11
5	56/M	9	3.06	90	2.1	18.9	4.0	12.4	1.26
6	54/M	2	2.36	65	0.2	2.2	1.3	9.9	1.02
7	69/F	3	1.77	90	1.6	11.7	2.0	8.3	1.09
8	63/M	2	1.69	71	2.2	8.3	4.8	5.6	0.98
9	57/F	3	2.76	69	1.8	7.6	15.6	3.9	1.01
10	72/M	4	2.67	68	0.7	4.0	4.0	5.3	0.96
Mean	66		2.40	75	1.9	10.3	7.9	6.9	1.05
SEM	±3		±0.19	±3	±0.4	±1.6	±2.1	±1.0	±0.03
Surgical controls (n=12)									
Mean	63		2.64	81	1.3	75.5	1.4	66.0	1.81
SEM	±3		±0.13	±1	±.2	±9.5	±.4	±11.0	±0.10
P value			NS	NS	NS	.001	.005	.001	.001

§ Onset of ARF defined by initial rise of serum creatinine concentration

* CI = cardiac index; MAP = mean arterial pressure; † FE_{Na} = fractional sodium excretion

$\dot{V}$ = urine flow rate; ‡ U/P_{osm} = urine to plasma osmolality; ♦ U/P_{IN} = urine to plasma inulin

ND = not determined; P value calculated from Student's t test for unpaired data; NS = not significant

TABLE 2

INULIN LEAKAGE AND CLEARANCES IN ARF

Patient	$\frac{1}{1-k_{in}}$ *	k_{in} †	Inulin Clearance "apparent"	Inulin Clearance true
			-----ml/min-----	
1	1.74	.43	11.7	20.4
2	1.79	.44	13.3	23.8
3	2.16	.54	14.5	31.5
4	1.98	.49	10.4	20.4
5	1.74	.43	18.9	33.2
6	3.6	.72	2.2	5.8
7	2.5	.60	11.7	29.3
8	1.53	.35	8.3	12.7
9	1.44	.31	7.6	10.9
10	2.29	.56	4.0	10.0
Mean	2.08	.49	10.3	19.8
SEM	±0.20	±.04	1.6	3.1

*$1/(1-k_{IN})$ = the limiting value of (Θ_{app}/Θ) vs Θ, and †k_I = fractional inulin leakage.

TABLE 3

COMPUTATION OF MEAN TUBULAR TRANSPORT COEFFICIENTS, k_D, AND RATIOS (k_D/k_{in}) FOR ARF PATIENTS

(k_{in} = 0.49 ± 0.04)

r Å	Θ_{app}/Θ	$1-k_D$	k_D	k_D/k_{in}
20	1.05	0.53	0.47	0.94
22	1.16	0.58	0.42	0.84
24	1.28	0.63	0.37	0.74
26	1.34	0.67	0.33	0.66
28	1.59	0.79	0.21	0.42
30	1.58	0.79	0.21	0.42
32	1.70	0.85	0.15	0.30
34	1.88	0.94	0.06	0.12
36	1.96	0.98	0.02	0.04
38	2.00	1.00	0.0	0.0
40	2.00	1.00	0.0	0.0

For abbreviations, see text.

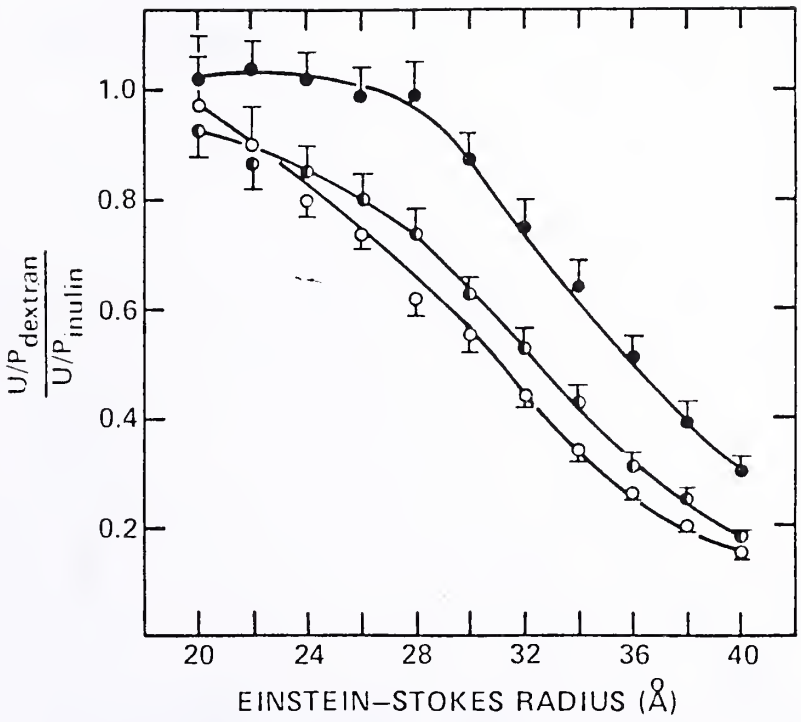


Figure 1: Fractional dextran clearance profile in 10 patients with ARF (•), in 7 normal controls (o), and in 12 post-cardiac surgical controls (⊙). All results are expressed as means and the standard error of the mean.

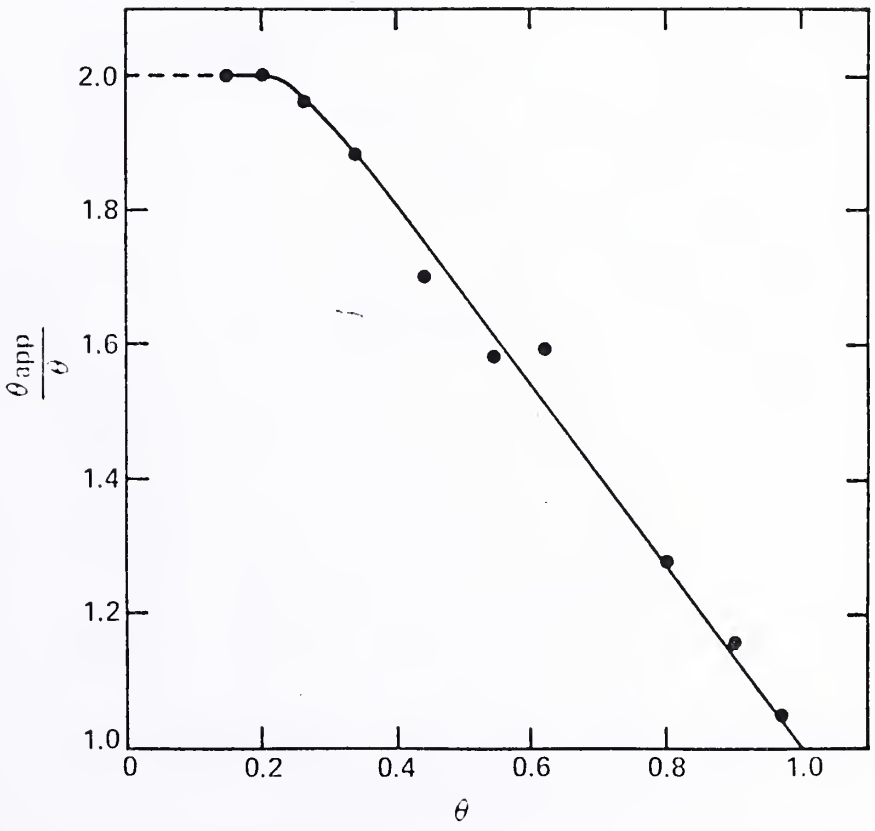


Figure 2: Mean fractional dextran clearance ratio (Θ_{app}/Θ) as a function of normal fractional dextran clearance (Θ).

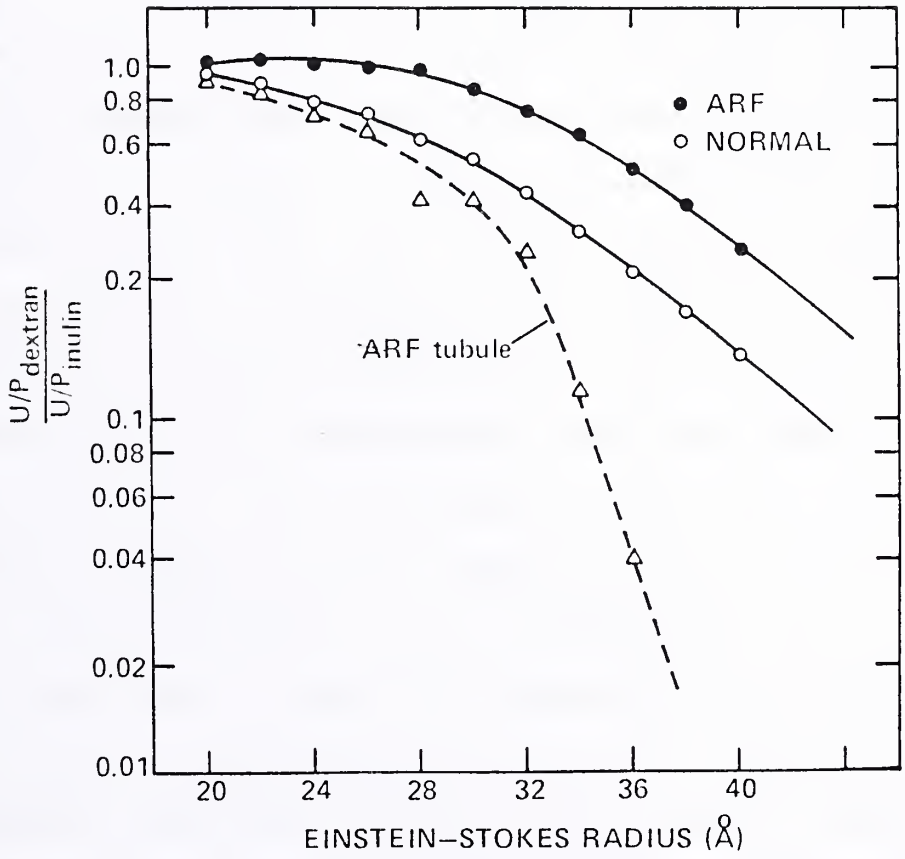


Figure 3: Fractional dextran clearance profiles are shown for the normal kidney (normal controls (o)), and the kidney in acute renal failure (•). The lower curve is the calculated dextran sieving curve for the damaged tubule in ARF (Δ).

IV. Discussion

In order to calculate fractional inulin backleak in ARF, we assumed that the fractional dextran clearance profile in Bowman's space in these patients resembles that in the urine of normal controls. At least three pathophysiological events in experimental animal ARF (and presumably in human ARF) could influence the transglomerular passage of macromolecules. Two of these changes, renal vasoconstriction(12) and a decline in glomerular transcapillary hydraulic pressure difference(17) are predicted to increase the permeation of dextrans with r 20 A into Bowman's space. That renal plasma flow was substantially depressed in our patients is suggested by an invariable and often severe depression of cardiac index at the time of each study (Table 1 and Refs. 61 & 73). Furthermore, low renal plasma flow has been demonstrated in other varieties of human ARF(45,75A). In addition, tubular obstruction, which appears to be an especially important pathogenetic mechanism in the postischemic model of experimental ARF, would tend to lower the glomerular transcapillary hydraulic difference and so increase the sieving of dextrans across the glomerular capillary wall(2,32,89,90). Recent evidence, however, indicates that a decline in the glomerular ultrafiltration coefficient occurs in both nephrotoxic and ischemic animal models of ARF(9,11,23,84). Assuming that the glomerular ultrafiltration coefficient also declines in human ARF, the transglomerular passage of macromolecules would be restricted, thus offsetting any increase in dextran sieving resulting from altered glomerular pressures and flows(17). Since the net effects of these opposing determinants of glomerular permselectivity to macromolecules

cannot be measured precisely in man, the use of the normal fractional dextran clearance profile can obviously provide only an approximation of fractional inulin backleak.

In postischemic models of unilateral ARF in the rat, inulin microinjection studies have, in general, indicated a direct relationship between the severity of the renal ischemia and the degree of transtubular inulin backleak. Negligible inulin backleak has followed prolonged partial renal ischemia or total renal ischemia of only 15 minutes duration(28,32); by contrast, only 36-58% of microinjected inulin was recovered in the urine from kidneys subjected to 60 min. of total ischemia(32,34,88). About 40% of nonrecovered inulin was excreted by the contralateral kidney in these studies, indicating substantial peritubular capillary uptake of inulin leaked through the damaged tubular wall. Given the indirect method we have used to calculate k_{IN}, it is interesting to note that our finding of a 31-72% transtubular leak of filtered inulin in this human variety of hemodynamically mediated ARF is in good agreement with that found following 60 min. of total renal ischemia in the rat. Similarly, our computed sieving characteristics of the damaged tubular wall are consistent with the findings from microinjection studies in post-ischemic experimental ARF, in which horseradish peroxidase (r=30 Å) was found to leak out of damaged tubules(32), while albumin (r=36 Å) was retained within the tubular lumen(88). Accordingly, our model appears to provide data in man consistent with those more directly obtained in experimental ARF in animals.

Glomerular filtration rate, when corrected for backleak in our patients with ARF, averaged 20 $ml.min^{-1}.1.73\ M^{-2}$, a value

corresponding to only 26% of that in cardiac surgical controls with similarly depressed cardiac performance. Therefore, additional mechanisms must have been operative to explain the profound depression of glomerular ultrafiltration. It is possible, for example, that renal vascular resistance may have been disproportionately elevated in ARF, resulting in a lower renal filtration fraction than in the cardiac surgical controls. In addition, a reduction in net glomerular ultrafiltration pressure (due to tubular obstruction) and/or glomerular ultrafiltration coefficient might be invoked to explain the reduced GFR. Since each of these observations in the determinants of glomerular ultrafiltration has been demonstrated in post-ischemic experimental ARF, it seems reasonable to assume that they provide the basis for that portion of GFR depression not accounted for by backleak in our patients.

V. Appendix

For the normal kidney, the rate of tubular inulin and dextran resorption is zero, and mass conservation requires that:

$$U_{in}V = GFR \cdot UF_{in} = GFR \cdot P_{in} \quad (A1)$$

$$U_{D_i}V = GFR \cdot UF_{D_i} = \Theta_{D_i} \cdot GFR \cdot P_{D_i} \quad (A2)$$

where $U_{in}V$ and $U_{D_i}V$ are the urine excretion rates of inulin and dextrans of ith molecular radius in mg/min, respectively; UF_{in} and UF_{D_i} refer to the concentration of these solutes in glomerular ultrafiltrate, and other symbols are defined as before. Since $UF_{in} = P_{in}$, it follows that the sieving coefficient (Θ) for the ith dextran is:

$$\Theta = (UF_{D_i} / P_{D_i}) = (U_{D_I} / U_{in}) / (P_{D_i} / P_{in}) \quad (A3)$$

For the kidney in ARF, however, if tubular backleak occurs and is assumed to be passive (i.e. driven solely by diffusion where the "sink" concentration is negligible), then

$$Leak_{in} = k_{in} \cdot GFR \cdot UF_{in} \quad (0 < k_{in} < 1.0) \quad (A4)$$

$$Leak_{D_i} = k_{D_i} \cdot GFR \cdot UF_{D_i} \quad (0 < k_{D_i} < 1.0) \quad (A5)$$

where $Leak_{in}$ and $Leak_{D_i}$ refer to the rates of transtubular backleak of inulin and dextran in mg/min, respectively, and k_{in} and k_{D_i} are the fractions of filtered inulin and dextran lost from the tubule.

The damaged tubular wall is presumably permeable to dextrans as well as to inulin, but the permeability should be a declining function (f) of dextran molecular size (r) that is:

$$k_{D_i} = f(r_{D_i}) \qquad (3)$$

$$k_{D_i} \to k_{in} \text{ as } r_{D_i} \to r_{in};\ k_{D_i} \to 0 \text{ as } r_{D_i} \to \infty$$

In this case mass-conservation requires that

$$U_{in}V = GFR \cdot P_{in}(1-k_{in}) \qquad (A6)$$

$$U_{D_i}V = \Theta_{D_i} \cdot GFR \cdot P_{D_i}(1-k_{D_i}) \qquad (A7)$$

and thus:

$$U_{D_i}V / U_{in}V = U_{D_i} / U_{in} = P_{D_i} / P_{in} \cdot \Theta\,[(1-k_{D_i})/(1-k_{in}) \qquad (A8)$$

If we now define the "apparent" dextran sieving coefficient as:

$$\Theta_{app} = (U_{D_i} / U_{in}) / (P_{D_i} / P_{in}) \qquad (A9)$$

then:

$$\Theta_{app} = \Theta\,[(1-k_{D_i}) / (1-k_{in})] = \Theta\,[1-f(r_{D_i})] / (1-k_{in}) \qquad (4)$$

TABLE I. FRACTIONAL DEXTRAN CLEARANCES IN PATIENTS WITH ACUTE RENAL FAILURE

Dextran r (Å)	20	22	24	26	28	30	32	34	36	38	40
Normal controls (Mean ± SE)	.97 ±.05	.90 ±.06	.80 ±.03	.74 ±.03	.62 ±.03	.55 ±.03	.44 ±.02	.34 ±.02	.26 ±.01	.20 ±.01	.15 ±.01
Cardiac surgical controls (Mean ± SE)	.93 ±.05	.87 ±.05	.85 ±.04	.80 ±.05	.74 ±.04	.63 ±.03	.53 ±.03	.43 ±.03	.31 ±.02	.25 ±.02	.18 ±.02
ARF Patients											
1	.99	1.10	1.09	1.07	.98	.85	.72	.61	.47	.34	.25
2	1.25	1.27	1.19	1.08	1.29	.91	.77	.62	.45	.32	.25
3	.92	.97	.89	.98	1.11	1.08	.91	.74	.57	.40	.29
4	.93	.99	1.05	1.04	1.02	.94	.78	.67	.54	.40	.31
5	1.17	1.26	1.30	1.15	.96	.84	.65	.55	.42	.40	.31
6	1.01	1.12	.99	1.22	1.25	1.18	1.13	1.01	.85	.68	.54
7	1.03	.93	1.01	.93	.96	.86	.78	.66	.55	.46	.36
8	.94	.83	.94	.85	.81	.72	.62	.52	.40	.31	.22
9	.87	.94	.97	.95	.86	.74	.62	.49	.36	.25	.17
10	1.09	1.00	.80	.68	.65	.60	.56	.55	.47	.42	.34
Mean	1.02	1.04	1.02	1.00	.99	.87	.75	.64	.51	.40	.30
± SE	.04	.05	.05	.05	.06	.05	.05	.05	.04	.04	.03
p* vs normal controls	NS	NS	<.005	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
p* vs cardiac surgical controls	NS	.05	<.02	<.02	<.005	<.001	<.001	<.001	<.001	<.01	<.005

p* values calculated from student's t test for unpaired data.

Fig 1. Calibration curve of Column A

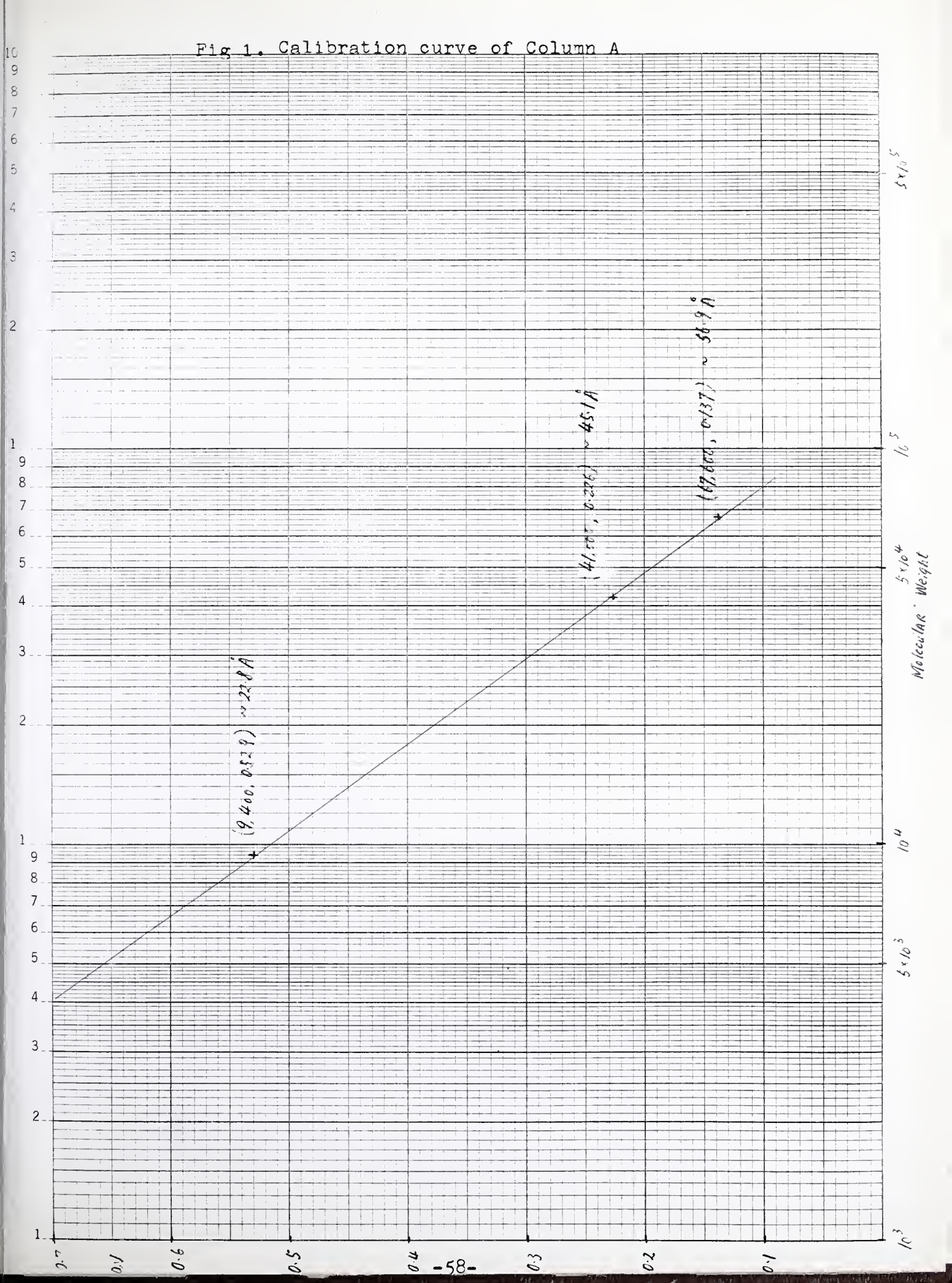


Fig. 2 Calibration curve of Column B

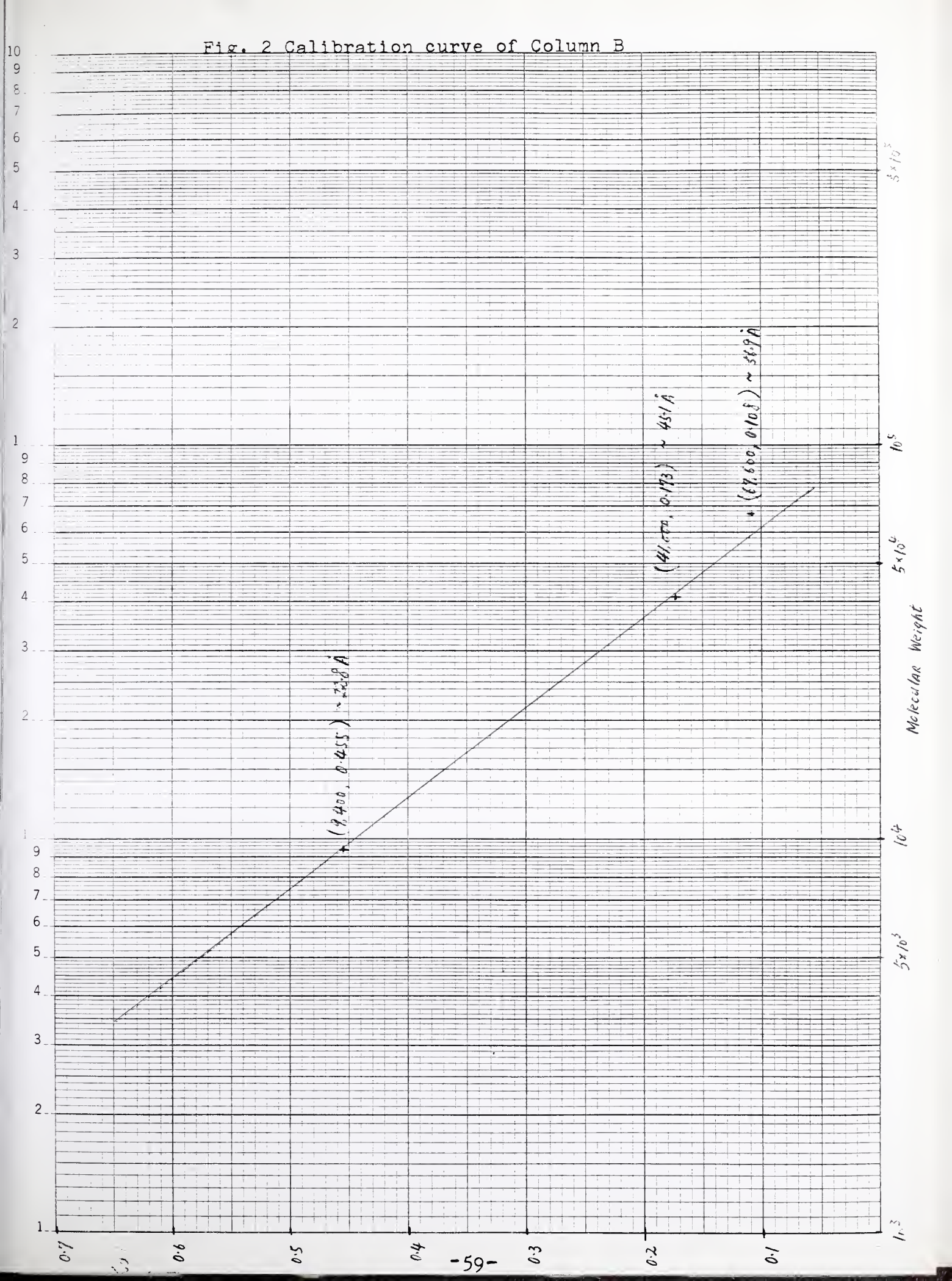


Fig. 3 Calibration Curve of Column C

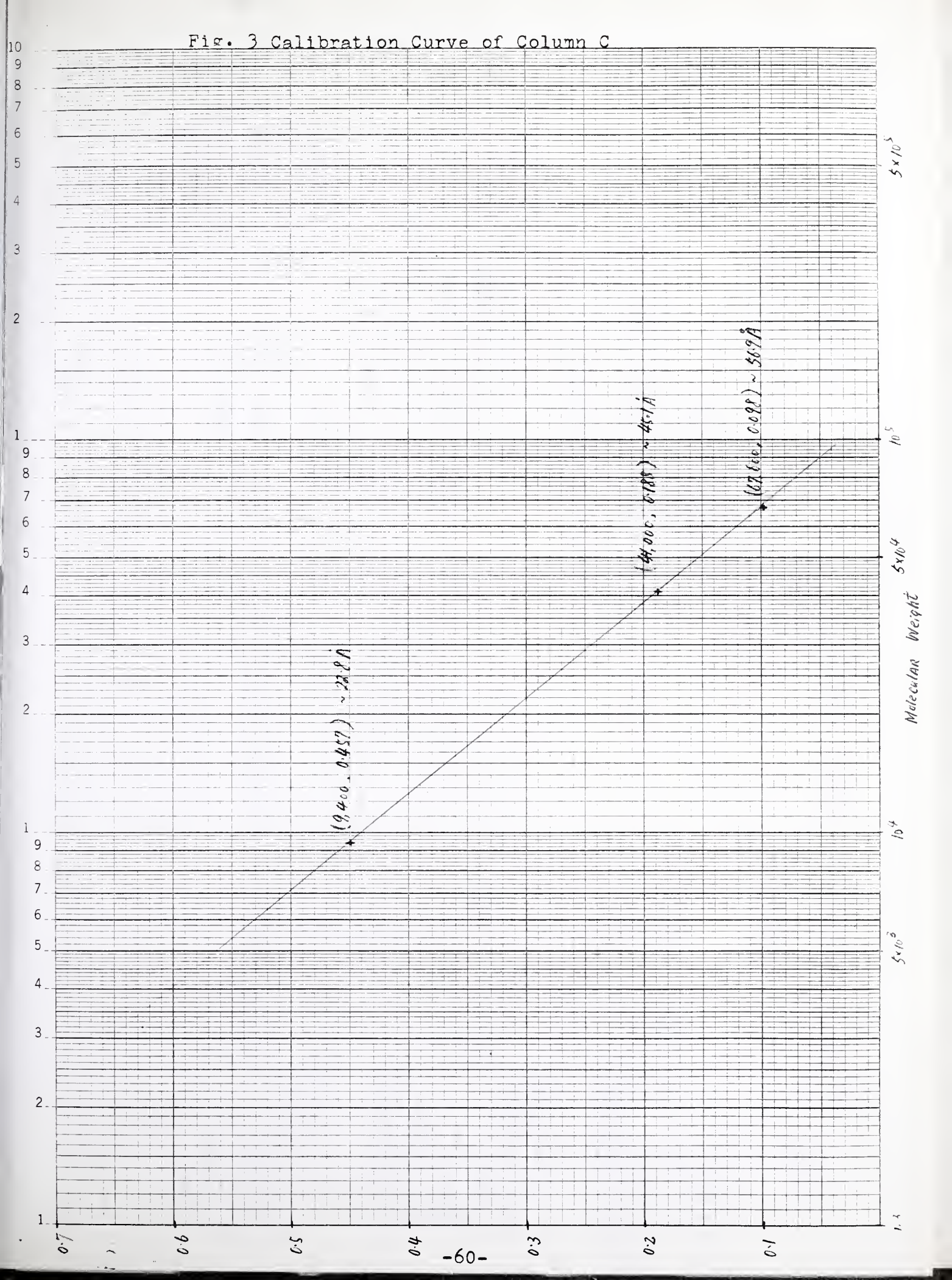
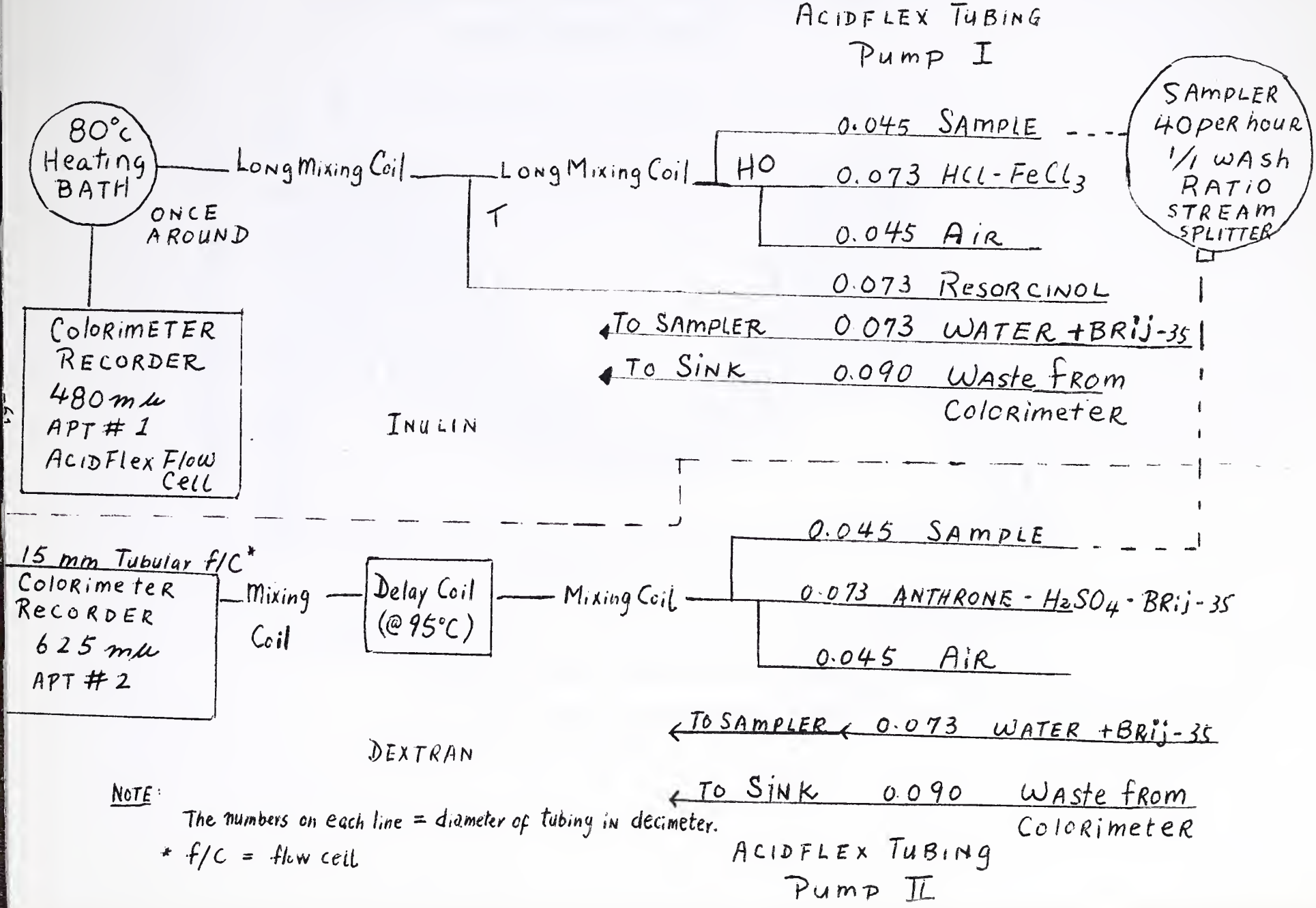


Fig 4 Flow Diagram of Inulin & Dextran Assay



NOTE:

The numbers on each line = diameter of tubing in decimeter.

* f/C = flow cell

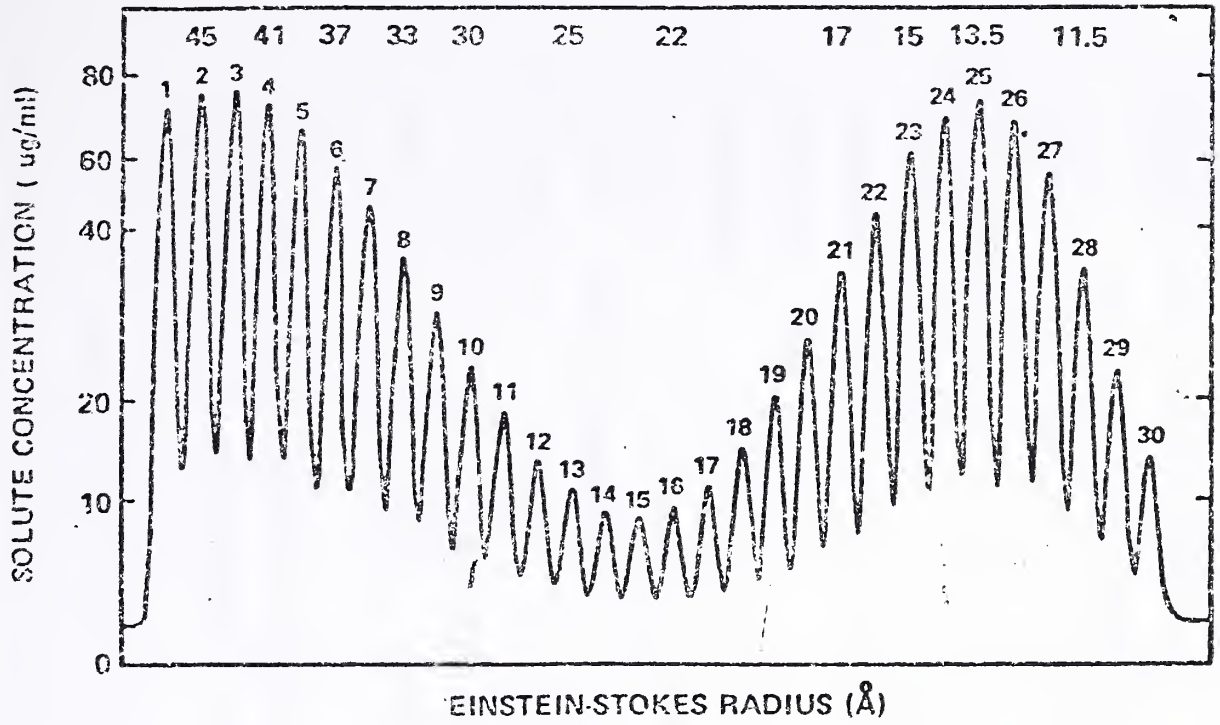


Fig. 5 Concentration profile of the above solution after chromatographic separation on the S-200 column into 30 eluted fractions and then subjected to the anthrone analysis (see text)

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